K950625

REVISED 510(k) SUMMARY

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DEVICE NAME: Ortho-mune™ OK-COMBO CD4-FITC/CD8-PE (OKT™4A/OKT8) Monoclonal Antibody (Murine)

PREDICATE: Simultest™ CD4/CD8 (Leu™-3a/2a)

DATE: February 16, 1996

DEVICE DESCRIPTION

Ortho-mune OK-COMBO CD4-FITC/CD8-PE (OKT4A/OKT8) Monoclonal Antibody (Murine) is a blend of the individual purified monoclonal antibodies OKT4A and OKT8 conjugated to the fluorochromes fluorescein isothiocyanate and phycoerythrin respectively.

INTENDED USE

Ortho-mune OK-COMBO CD4-FITC/CD8-PE is intended for use in identification and enumeration of CD4+ and CD8+ human T lymphocytes in whole blood by flow cytometry. The intended use is the same as the intended use of the predicate device, Simultest CD4/CD8 (Leu-3a/2a) commercially distributed by Becton Dickinson Immunocytometry Systems.

TECHNOLOGICAL CHARACTERISTICS

Both Ortho-mune OK-COMBO CD4-FITC/CD8-PE (OKT4A/OKT8) Monoclonal Antibody (Murine) and Simultest CD4/CD8(Leu-3a/2a) utilize monoclonal antibodies specific for human helper/inducer T cells (OKT4A/Leu 3a) and human suppressor/cytotoxic T cells (OKT8/Leu-2a) respectively, conjugated to the same fluorochromes, fluorescein isothiocyanate and phycoerythrin.

PERFORMANCE CHARACTERISTICS

Performance of the two color reagent Ortho-mune OK-COMBO CD4-FITC/CD8-PE (OKT4A/OKT8) Monoclonal Antibody (Murine) was compared with that of Simultest CD4/CD8 (Leu-3a/2a) at three external, geographically distinct sites. Whole blood specimens from 190 normal donors, and 83 AIDS/ARC patients were stained and analyzed using the ORTHO CYTORONABSOLUTE™ flow cytometer, Ortho Diagnostic Systems Inc.

For each specimen, the percentage of gated cells which showed positive by each marker was calculated. The mean and range of the percent CD4+ and percent CD8+ cells for the normal donor and AIDS/ARC population are shown in Table 1 and Table 2 respectively.

TABLE 1

PERCENT POSITIVE STAINED CELLS IN NORMAL DONORS DETECTED BY OKT4A/OKT8 AND LEU-3a/2a ASSAYED ON THE CYTORONABSOLUTE N=190

Ortho-mune Reagent	Mean %	Range %	BD Reagent	Mean %	Range %
CD4+ (OKT4A)	47.5	11.6-70.8	CD4+ (LEU3a)	44.1	13.1-61.2
CD8+ (OKT8)	27.8	10.9-72.8	CD8+ (LEU2a)	29.5	12.5-69.1

TABLE 2

PERCENT POSITIVE STAINED CELLS IN AIDS/ARC PATIENTS DETECTED BY OKT4A/OKT8 AND LEU-3a/2a ASSAYED ON THE CYTORONABSOLUTE N=83					
Ortho-mune Reagent	Mean %	Range %	**BD Reagent**	Mean %	Range %
CD4+ (OKT4A)	18.2	0.1-60.4	CD4+ (LEU3a)	15.8	0.2-50.4
CD8+ (OKT8)	56.1	12.6-81.9	CD8+ (LEU2a)	58.9	22.5-88.4

Linear regression analysis of total percent CD4+ cells from the combined normal and AIDS/ARC populations is found in Chart 1. Likewise, linear regression analysis of percent CD8+ cells from the combined normal and AIDS/ARC populations is found in Chart 2.

CHART 1

OK-COMBO CD4/CD8 vs Simultest Leu-3a/2a

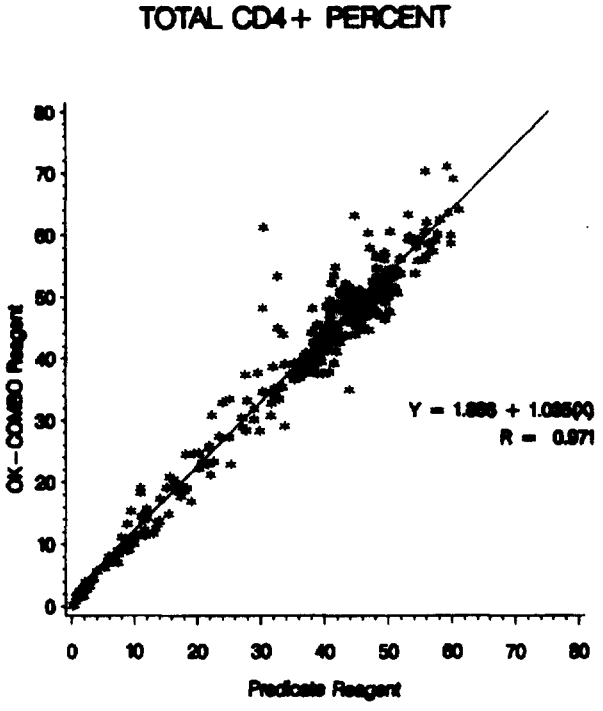
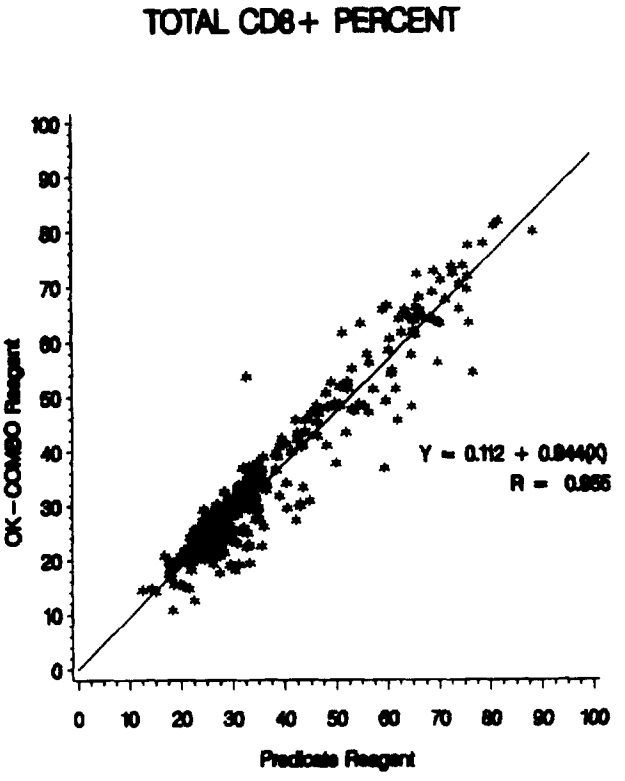


CHART 2

OK-COMBO CD4/CD8 vs Simultest Leu-3a/2a



These studies demonstrate that the performance of the two color reagent Ortho-mune OK-COMBO CD4-FITC/CD8-PE (OKT4A/OKT8) Monoclonal Antibody (Murine) is equivalent to Simultest CD4/CD8 (Leu-3a/2a) reagent in identification and enumeration of CD4+ and CD8+ human lymphocytes in whole blood by flow cytometry.

Reproducibility studies were performed at three independent laboratories using samples with low, normal, and high relative percent CD4+ and CD8+ cells.

Specimens from each of eleven normal donors (whole blood, EDTA) were processed using monoclonal antibodies bound to microbeads to produce samples of low, normal, and high relative percent CD4+ and CD8+ cells. The samples were separated into aliquots for each laboratory. Samples were stained in replicates of 10 with Ortho-mune OK-COMBO CD4/CD8 reagent and analyzed using the ORTHO CYTORONABSOLUTE flow cytometer.

For within laboratory reproducibility, the variance for the replicate results was calculated within site, concentration and donor. The variance was averaged across site, concentration and donor. The square root replicate variance (SD) was divided by the appropriate mean percent positive result (by site and concentration) and multiplied by 100 to obtain the CV. Within laboratory reproducibility results for determination of total percent CD4+ and percent CD8+ cells are presented in Table 3.

TABLE 3

WITHIN LABORATORY REPRODUCIBILITY OK-COMBO CD4/CD8							
N = 11 donors							
OK-COMBO CD4/CD8	All SITES Mean Percent Positive	Site A		Site B		Site C	
		CV	# Reps	CV	# Reps	CV	# Reps
TOTAL $CD4^+$ Low	11.232	8.268	98	7.358	109	6.701	109
TOTAL $CD4^+$ Normal	49.455	3.118	110	2.443	110	2.736	109
TOTAL $CD4^+$ High	67.528	3.049	110	2.093	110	3.360	104
TOTAL $CD8^+$ Low	7.521	15.735	110	7.186	110	7.972	104
TOTAL $CD8^+$ Normal	32.782	4.244	110	3.102	110	3.442	109
TOTAL $CD8^+$ High	56.234	3.392	98	2.462	109	2.740	109

The between laboratory CV was computed as follows. The mean percent positive for each site within concentration was calculated. The SD was computed on the three site means within concentration and the CV was obtained by dividing the SD by the overall mean within concentration and multiplying by 100. Between laboratory reproducibility results for determination of total percent CD4+ and percent CD8+ cells are presented in Table 4.

TABLE 4

BETWEEN LABORATORY REPRODUCIBILITY OK-COMBO CD4 /CD8 N = 11 donors					
	SITE A	SITE B	SITE C	ACROSS SITE	
OK-COMBO CD4/CD8	Mean Percent Positive (All Donors)	Mean Percent Positive (All Donors)	Mean Percent Positive (All Donors)	Coefficient of Variation	# Reps
TOTAL $CD4^+$ Low	10.744	11.994	11.389	5.496	316
TOTAL $CD4^+$ Normal	50.103	49.392	48.937	1.189	329
TOTAL $CD4^+$ High	67.934	68.473	66.235	1.729	324
TOTAL $CD8^+$ Low	7.908	7.623	7.097	5.453	324
TOTAL $CD8^+$ Normal	32.636	32.913	32.741	0.427	329
TOTAL $CD8^+$ High	55.979	56.787	55.271	1.354	316

Ortho-mune OK-COMBO CD4/CD8 immunophenotyping reagent shows acceptable within and between laboratory reproducibility for determination of total CD4+ and CD8+ lymphocyte percentages

A linearity study was performed using an automated hematology analyzer to determine total lymphocyte count, and the CYTORONABSOLUTE flow cytometer to determine the percent positive CDx cells.

Specimens from four normal donors (whole blood, EDTA) were processed to produce samples with low, normal and high numbers of lymphocyte subsets. Each whole blood specimen was concentrated by harvesting the buffy coat to obtain a white blood cell count between 20,000 and 40,000 cells/ul and then diluting to produce samples of high, normal and low numbers of lymphocyte subsets. A portion of each sample was stained in triplicate using Ortho-mune OK-COMBO CD4/CD8 immunophenotyping reagent and analyzed using the CYTORONABSOLUTE flow cytometer. The total lymphocyte count of the concentrated sample for each donor was obtained using an automated hematology analyzer.

Linear regression analyses were performed as follows. The expected (X axis) values were calculated by multiplying the corresponding serial dilutions by the hematology analyzer derived buffy coat lymphocyte count and by the CYTORONABSOLUTE derived lymphocyte subset percent positive. The observed (Y axis) values were determined as the total lymphocyte count calculated from the hematology derived value of the concentrated sample times the CYTORONABSOLUTE derived lymphocyte subset percent positive at each dilution.

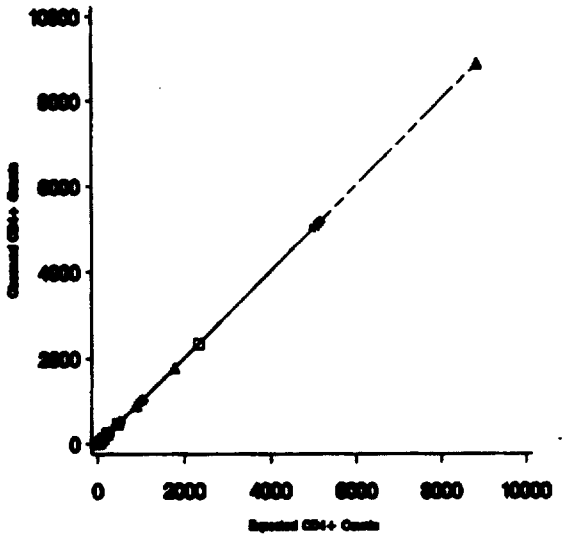
The OK-COMBO CD4/CD8 reagent demonstrated linear performance for both total CD4+ and CD8+ lymphocyte subsets across a lymphocyte count range of 20 cells/ul to 18,676 cells/ul as demonstrated with slopes indistinguishable from 1 and R values of 1.000.

Linear regression analyses of observed versus expected values for total percent CD4+ and percent CD8+ cells for each donor specimen are shown in Chart 3 and Chart 4 respectively. Regression analysis statistics are provided in Table 5.

CHART 3

OK–COMBO CD4/CD8

CD4+

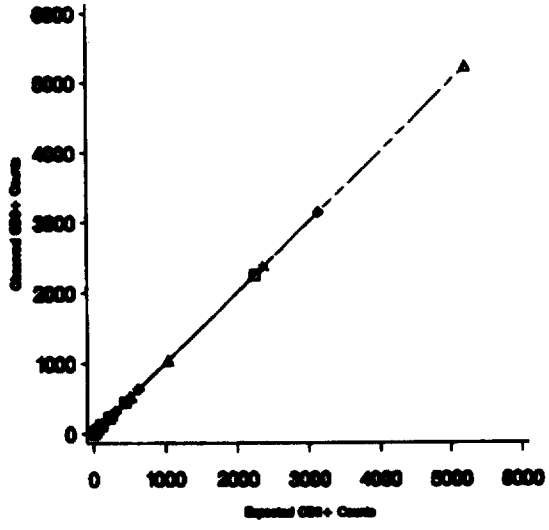


DONOR ⟷ 71 ⟷ 72 ⟷ 73 ⟷ 74

CHART 4

OK–COMBO CD4/CD8

CD8+



DONOR ⟷ 71 ⟷ 72 ⟷ 73 ⟷ 74

TABLE 5

OK-COMBO CD4/CD8	Donor	SLOPE	CI	INTERCEPT	CI	R
TOTAL CD4	1	0.998	0.004	8.660	6.795	1.000
TOTAL CD4	2	1.000	0.002	-0.558	1.592	1.000
TOTAL CD4	3	0.999	0.003	4.117	2.719	1.000
TOTAL CD4	4	0.999	0.003	11.075	9.120	1.000
TOTAL CD4	All	0.999	0.001	5.325	2.743	1.000
TOTAL CD8	1	0.999	0.003	2.389	2.334	1.000
TOTAL CD8	2	0.999	0.003	1.547	2.374	1.000
TOTAL CD8	3	1.000	0.004	2.859	4.226	1.000
TOTAL CD8	4	1.000	0.003	5.326	5.797	1.000
TOTAL CD8	All	0.999	0.001	2.943	1.553	1.000

CONCLUSION

Performance of the two color reagent Ortho-mune OK-COMBO CD4-FITC/CD8-PE (OKT4A/OKT8) Monoclonal Antibody (Murine) is equivalent to Becton Dickinson Simultest CD4/CD8 (Leu-3a/2a) reagent for identification and determination of percent CD4+ and CD8+ lymphocytes in whole blood by flow cytometry.